ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Атипичен ювенилен паркинсонизъм
Определение на заболяването
Атипичният ювенилен паркинсонизъм е комплексна форма на Паркинсонова болест с ранно начало. Клинично се манифестира с пирамидни белези, абнормни очни движения, психиатрична симптоматика (депресия, тревожност, медикаментозно-индуцирана психоза и нарушения на импулсивния контрол), когнитивни нарушения и други неврологични симптоми (като атаксия и епилепсия) наред с класически паркинсонови симптоми.
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G20
Код на заболяването по Orpha code
ORPHA391411
Епидемиологични данни за заболяването в Република България
Заболеваемост и болестност неизвестни. Предполага се болестност <1/1 000 000 сходна на останалите страни в Европейския съюз.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Milanov I, Kmetska K, Karakolev B, Nedialkov E. Prevalence of Parkinson's disease in Bulgaria. Neuroepidemiology. 2001;20(3):212-4. 2. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., Elpeleg, O. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7: e36458, 2012. 3. Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H., Tolun, A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19: 320-324, 2013. 4. Olgiati, S., Quadri, M., Fang, M., Rood, J. P. M. A., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., Zhang, J., The International Parkinsonism Genetics Network, and 10 others. DNAJC6 mutations associated with early-onset Parkinson's disease. Ann. Neurol. 79: 244-256, 2016. 5. Krebs, C. E., Karkheiran, S., Powell, J. C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R. H., Shahidi, G. A., Buxbaum, J. D., De Camilli, P., Yue, Z., Paisan-Ruiz, C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34: 1200-1207, 2013.

Епидемиологични данни за заболяването в Европейския съюз
<1/1 000 000; До момента има публикувани само няколко семейства в целия свят вкл. и в Европейския съюз.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., Elpeleg, O. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7: e36458, 2012. 2. Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H., Tolun, A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19: 320-324, 2013. 3. Olgiati, S., Quadri, M., Fang, M., Rood, J. P. M. A., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., Zhang, J., The International Parkinsonism Genetics Network, and 10 others. DNAJC6 mutations associated with early-onset Parkinson's disease. Ann. Neurol. 79: 244-256, 2016. 4. Krebs, C. E., Karkheiran, S., Powell, J. C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R. H., Shahidi, G. A., Buxbaum, J. D., De Camilli, P., Yue, Z., Paisan-Ruiz, C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34: 1200-1207, 2013.
Оценка на съответствието на заболяването с дефиницията за рядко заболяване съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето
Заболяване е с разпространение под 5/10 000 души от населението на Европейския съюз.
Критерии за диагностициране на заболяването
Диагностициране на заболяването (дефиниция на случай): Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест. Признаците и симптомите на заболяването: Заболяването е с ювенилно начало (обикновено в началото на юношеството или двайсетте години). Клиничната изява включва пирамидни белези, дистония, психична симптоматика (депресия, тревожност, медикаментозно-индуцирана психоза, деменция и нарушен контрол на импулсивността) на фона на класически паркинсонови симптоми (брадикинезия, постурална нестабилност, ригидност, неволеви движения, дизартрия, супрануклеарна пареза, хипомимия, нарушения в походката и миоклонични потрепвания). Наблюдават се също когнитивни нарушения. PARK19A се характеризира с начало на паркинсонизма в първото или второто десетилетие. Някои пациенти имат допълнителни неврологични признаци вкл. ментална ретардация и припадъци. PARK19B е с начало на паркинсонизма между трето и четвърто десетилетие. Заболяването прогресира бавно, като показва черти подобни на класическата с късно начало Паркинсонова болест с добър отговор на допаминергична терапия. PARK 20 се характеризира с развитие на паркинсонизъм в началото на зрелостта. В допълнение пациентите могат да имат припадъци, когнитивно влошаване, абнормни очни движения и дистония.

Етиологията и патогенезата: Независимо един от друг и едновременно Krebs и колеги (2013) и Quadri и колеги (2013) идентифицират хомозиготна мис мутация на SYNJ1 гени (R258Q; 604297.0001) при PARK20. Данните от Krebs и колеги (2013) и Quadri и колеги (2013) предполагат, че дефектно рециклиране и транспорт на синаптичните везикули може да играе роля в невродегенеративния процес при Паркинсонова болест.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., Elpeleg, O. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7: e36458, 2012.
3. Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H., Tolun, A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19: 320-324, 2013.
4. Olgiati, S., Quadri, M., Fang, M., Rood, J. P. M. A., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., Zhang, J., The International Parkinsonism Genetics Network, and 10 others. DNAJC6 mutations associated with early-onset Parkinson's disease. Ann. Neurol. 79: 244-256, 2016.
5. Krebs, C. E., Karkheiran, S., Powell, J. C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R. H., Shahidi, G. A., Buxbaum, J. D., De Camilli, P., Yue, Z., Paisan-Ruiz, C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34: 1200-1207, 2013.
6. Quadri, M., Fang, M., Picillo, M., Olgiati, S., Breedveld, G. J., Graafland, J., Wu, B., Xu, F., Erro, R., Amboni, M., Pappata, S., Quarantelli, M., and 9 others. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset parkinsonism. Hum. Mutat. 34: 1208-1215, 2013.

Алгоритми за диагностициране на заболяването

Алгоритми за диагностициране на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, а на когнитивните нарушения съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Анамнезата: Заболяването е с ювенилно начало (обикновено в началото на юношеството или двайсетте години). Клиничната изява включва пирамидни белези, дистония, психична симптоматика (депресия, тревожност, медикаментозно-индуцирана психоза, деменция и нарушен контрол на импулсивността) на фона на класически паркинсонови симптоми (брадикинезия, постурална нестабилност, ригидност, неволеви движения, дизартрия, супрануклеарна пареза, хипомимия, нарушения в походката и миоклонични потрепвания). Наблюдават се също когнитивни нарушения.

PARK19A се характеризира с начало на паркинсонизма в първото или второто десетилетие. Някои пациенти имат допълнителни неврологични признаци вкл. ментална ретардация и припадъци.

PARK19B е с начало на паркинсонизма между трето и четвърто десетилетие. Заболяването прогресира бавно, като показва черти подобни на класическата с късно начало Паркинсонова болест с добър отговор на допаминергична терапия.
PARK 20 се характеризира с развитие на паркинсонизъм в началото на зрелостта. В допълнение пациентите могат да имат припадъци, когнитивно влошаване, абнормни очни движения и дистония.
Диференциалната диагноза на заболяването: болест Уилсън и др.
Лабораторни, образни и хистологични изследвания: MRT на глава обичайно е нормален. PET/SPEKT показват нигростриатални абнормности, типични за Паркинсонова болест.
Генетични изследвания и медико-генетично консултиране: Атипичният ювенилен паркинсонизъм обичайно е с автозомно-рецесивно унаследяване, като повече случаи са при кръвна връзка между родителите. Има съобщения и за спорадични случаи. Мутации в гените: ATP13A2 ген на хромозома 1p36 (PARK9), PLA2G6 ген на хромозома 22q13.1 (PARK14), FBXO7 ген на хромозома 22q12.3 (PARK15), DNAJC6 ген на хромозома 1p31.3(PARK19A; PARK19B), SPG11 (15q13-q15), SPG15 (14q24.1) и SYNJ1 ген на хромозома 21q22.2 (PARK20) се асоциират с атипичния ювенилен паркинсонизъм.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
3. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., Elpeleg, O. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7: e36458, 2012.
4. Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H., Tolun, A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19: 320-324, 2013.
5. Olgiati, S., Quadri, M., Fang, M., Rood, J. P. M. A., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., Zhang, J., The International Parkinsonism Genetics Network, and 10 others. DNAJC6 mutations associated with early-onset Parkinson's disease. Ann. Neurol. 79: 244-256, 2016.
6. Krebs, C. E., Karkheiran, S., Powell, J. C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R. H., Shahidi, G. A., Buxbaum, J. D., De Camilli, P., Yue, Z., Paisan-Ruiz, C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34: 1200-1207, 2013.

Алгоритми за лечение на заболяването

Алгоритми за лечение на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, а на когнитивните нарушения съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Терапевтичните подходи към заболяването, в това число консервативни и оперативни, техните предимства, рискове и очаквана ефективност: При пациентите с PARK 20 и PARK 19B се съобщава за добър ефект от допаминергична терапия, въпреки че се развиват леводопа-индуцирани дискинезии. При един случай също е съобщено за забележимо подобрение от дълбока мозъчна стимулация. При PARK19A се съобщава както за случаи на добър ефект от допа-терапия (но развитие на странични ефекти), така и за случаи на липса на ефект.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
3. Olgiati, S., Quadri, M., Fang, M., Rood, J. P. M. A., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., Zhang, J., The International Parkinsonism Genetics Network, and 10 others. DNAJC6 mutations associated with early-onset Parkinson's disease. Ann. Neurol. 79: 244-256, 2016.
4. Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H., Tolun, A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19: 320-324, 2013.
5. Krebs, C. E., Karkheiran, S., Powell, J. C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R. H., Shahidi, G. A., Buxbaum, J. D., De Camilli, P., Yue, Z., Paisan-Ruiz, C. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34: 1200-1207, 2013.
6. Quadri, M., Fang, M., Picillo, M., Olgiati, S., Breedveld, G. J., Graafland, J., Wu, B., Xu, F., Erro, R., Amboni, M., Pappata, S., Quarantelli, M., and 9 others. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset parkinsonism. Hum. Mutat. 34: 1208-1215, 2013.

Алгоритми за проследяване на заболяването

Алгоритми за проследяване на заболяването (необходимостта от последващи болнични и извънболнични грижи): Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Прогнозата на заболяването: При пациентите с добър отговор на допаминергичната терапия се наблюдава бавна и постепенна прогресия на заболяването над десетилетие.

Необходимостта от консултации с други специалисти: психиатър (при психиатрични симптоми);

Възможни усложнения: медикаментозно-индуцирани дискинезии, медикаментозно-индуцирана психоза, силно затруднена до невъзможна самостоятелна походка, когнитивни нарушения, заболявания, свързани с обездвижване, психиатрична симптоматика, гърчове.

Честота и тежест на усложненията и др.: често се наблюдават медикаментозно индуцирани дискинезии, като с напредване на заболяването нараства обездвижването и усложненията свързани с него.

В т.ч. научни публикации от последните пет години и приложена

библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015. 3. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., Elpeleg, O. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7: e36458, 2012.
Алгоритми за рехабилитация на заболяването
Алгоритми за рехабилитация на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
Необходими дейности за профилактика на заболяването (ако такива са приложими)
Дейности за профилактика на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба
Създаването на Национален експертен център „Редки невродегенеративни заболявания, протичащи с когнитивни, поведенчески и моторни нарушения" за диагностика, лечение и проследяване и рехабилитация включително и на пациенти с това заболявания под ръководството на чл.кор.проф.д-р Л. Трайков, дмн (национален експерт с най-голям опит и принос за диагностиката и лечението на тези заболявания).
Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)
Опитът на кандидатстващия експертен център под ръководството на чл. кор. проф.Трайков за диагноза и лечение на редки заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, датира от 2001 година със създаване на център за диагноза и лечение на невродегенеративни заболявания, протичащи с деменция и допълнително на център за диагноза и лечение на Паркинсонова болест. От дълги години този център е реферирал център за заболявания, протичащи с

паркинсонизъм с и без когнитивни нарушения, особено за комплексни, редки и наследствени случаи. През годините вследствие на натрупания опит и труд, както и значителен брой на пациенти с тези редки заболявания, реферирани към центъра са осъществени няколко дисертации в областта: 1. Когнитивни нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Мария Петрова, 2010 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Лонгитудинално проследяване на когнитивните нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Явор Желев, 2012 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. Клинико-генетични корелации при невродегенеративни заболявания, протичащи с паркинсонизъм (защитена дисертация за доктор по медицина от д-р Радка Павлова, 2013 г., ръководител: чл.-кор. проф. Лъчезар Трайков). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, протичащи с паркинсонизъм с и без когнитивен дефицит с подробно фенотипизиране на всеки един случай, което дава възможност за добър мониторинг на пациентите, както и изследователски анализ върху характеристиката на отделните заболявания. Дейността на центъра по отношение на диагноза и лечение на редки заболявания, протичащи с моторни и когнитивни нарушения, обхваща всички диагностични дейности съобразно новите диагностични критерии на тези заболявания, включително допълнителни изследвания, които са нужни за диференциална диагноза на атипични/ранни/наследствени случаи, включващи изследвания за биомаркери, невроизобразяващи и генетични фактори.

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A Deleterious Mutation in *DNAJC6* Encoding the Neuronal-Specific Clathrin-Uncoating Co-Chaperone Auxilin, Is Associated with Juvenile Parkinsonism

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Abstract

Parkinson disease is caused by neuronal loss in the substantia nigra which manifests by abnormality of movement, muscle tone, and postural stability. Several genes have been implicated in the pathogenesis of Parkinson disease, but the underlying molecular basis is still unknown for ~70% of the patients. Using homozygosity mapping and whole exome sequencing we identified a deleterious mutation in *DNAJC6* in two patients with juvenile Parkinsonism. The mutation was associated with abnormal transcripts and marked reduced DNAJC6 mRNA level. *DNAJC6* encodes the HSP40 Auxilin, a protein which is selectively expressed in neurons and confers specificity to the ATPase activity of its partner Hcs70 in clathrin uncoating. In Auxilin null mice it was previously shown that the abnormally increased retention of assembled clathrin on vesicles and in empty cages leads to impaired synaptic vesicle recycling and perturbed clathrin mediated endocytosis. Endocytosis function, studied by transferring uptake, was normal in fibroblasts from our patients, likely because of the presence of another J-domain containing partner which co-chaperones Hsc70-mediated uncoating activity in non-neuronal cells. The present report underscores the importance of the endocytic/lysosomal pathway in the pathogenesis of Parkinson disease and other forms of Parkinsonism.

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Introduction

Parkinson's disease (PD) is an insidious and progressive neurodegenerative disorder causing slowed movement, tremor, rigidity and postural instability. The disease is characterized by neuronal loss in the substantia nigra and other brain regions, and is usually associated with the formation of intracellular protein inclusions in damaged neurons, known as Lewy bodies. Several genes known to function in the endocytic/lysosomal pathway or in mitochondrial repair/elimination machinery have been implicated in the pathogenesis of PD. At present, known Mendelian forms and genetic risk factors of PD explain only about 30% of the disease risk at the general population level [1]. While familial forms of PD and Juvenile variants are rare, the identification of their disease-causing genes is important as they highlight specific pathways and because common genetic variants in these genes may confer a risk of developing the sporadic disease. Here, we report a homozygous mutation in *DNAJC6* in two patients with autosomal-recessive juvenile Parkinsonism.

Results

In order to localize the mutated gene in this family we searched for homozygous regions common to the two patients but not to their healthy brother, by genotyping dense DNA SNP arrays. This analysis resulted in identification of eight homozygous genomic regions of more than 2 Mb each, totaling 102.75 Mb. These regions encompass about 800 protein-coding genes, making the identification of plausible candidate genes difficult. We therefore performed whole exome sequencing of patient II-2 sample. This analysis resulted in the identification of 18,494 coding variants (single-nucleotide variants and small insertions and deletions, of which 7,387 variants were homozygous, but only 740 homozygous coding or splice site variants were present in the eight homozygous regions. Thirty variants were not annotated in dbSNP132, in the 1,000-genome or in our in-house database, and 15 remained after filtering out synonymous changes. Sanger sequencing confirmed only 11 changes and these segregated with the disease within the family. However, out of the 11 variants, ten were annotated in dbSNP135. We further checked for their conservation score

GERP (obtained via SeattleSeq Annotation website). The score of six variants was above 3.0 and these were tested for their potential pathogenicity using Polyphen, SIFT, and Mutation taster software. Three variants were reported by these tools as potentially pathogenic: Arg141Cys mutation in *POLR1C* (rs148385032), Cys3346Arg in *PKHD1* (rs149798764), and c.801 −2 A>G mutation in *DNAJC6* (at chr.1:65623981). Mutations in *POLR1C* were recently shown to cause Treacher Collins syndrome [2] and *PKHD1* mutations are associated with polycystic kidney and hepatic disease [3] and were thus excluded as candidate genes for PD. Of note the index case had normal kidneys as per abdominal ultrasound and did not display the facial characteristics of Treacher Collins syndrome. The c.801-2 A−>G mutation in the *DNAJC6* gene segregated with the disease state within the family; both patients were homozygous, while the parents and two healthy siblings were heterozygous for the mutation; one sister was homozygous for the normal allele (figure 1A–C). The mutation was not carried by any of 208 anonymous ethnic matched controls, neither was it present in the data of the 5379 Exomes available at the NHLBI Exome Sequencing Project website Release Version: v.0.0.9.

DNAJC6 encodes Auxilin which belongs to the evolutionarily conserved DNAJ/HSP40 family of proteins [4]. These proteins regulate molecular chaperone function by stimulating ATPase activity in many cellular processes and Auxilin functions specifically in the Clathrin Mediated Endocytosis (CME) pathway (figure 2). *DNAJC6* consists of 19 exons which encode 970 amino acids. The effect of the mutation on cDNA was studied in RNA from the lymphoblast cell line and from cultured skin fibroblasts of patient II-4. Homozygosity for the c.801 −2 A−>G mutation resulted in the generation of two mis-spliced cDNA transcripts; an in-frame exon 7-skipped transcript lacking amino acids 268–328, and an out-of-frame transcript with an insertion of the last 91 nucleotides of IVS 6 (c.801 −91) between exon 6 and 7, resulting in the addition of eight non-synonymous residues before reaching a termination codon. Importantly, the normally-spliced transcript was undetectable (figure 1E–F). This is suggestive but does not confirm pathogenicity of the c.801 −2 A−>G mutation within the family. Nonetheless, the ratio of *DNAJC6* cDNA normalized to beta-actin cDNA of five controls was 0.064+/−0.007 whereas the ratio was 0.011 in the patient, indicating a significant instability of the DNAJC6 mRNA in the patient's cells.

Discussion

CME is a major pathway for the internalization of selected protein cargo from the plasma membrane to membrane-bound internal compartments. The cargo, mainly receptors and their bound ligands, is recruited to nascent clathrin-coated pits, which then mature, invaginate, and ultimately undergo fission to produce Clathrin-Coated Vesicles (CCVs) [5,6]. Once the vesicle has pinched off from the plasma membrane, the coat is lost and its components are recycled. The vesicle itself then fuses with the membrane of a target compartment. The shedding of the coat, which occurs almost immediately after the endocytic CCV buds from the plasma membrane, is driven by ATP hydrolysis and is important for clathrin recycling [7,8]. The necessary ATPase activity is contributed by Hsc70, which like all Hsp70 homologues, interacts with J-domain containing cochaperones that specify its targets. In the case of clathrin coats, the relevant J-domain protein is Auxilin [9]. Three functional domains are instrumental for Auxilin's co-chaperone activity: an N-terminal PTEN-like domain (residues 40–421), which is important for recruitment of Auxilin onto CCVs, a middle domain which binds clathrin, and a

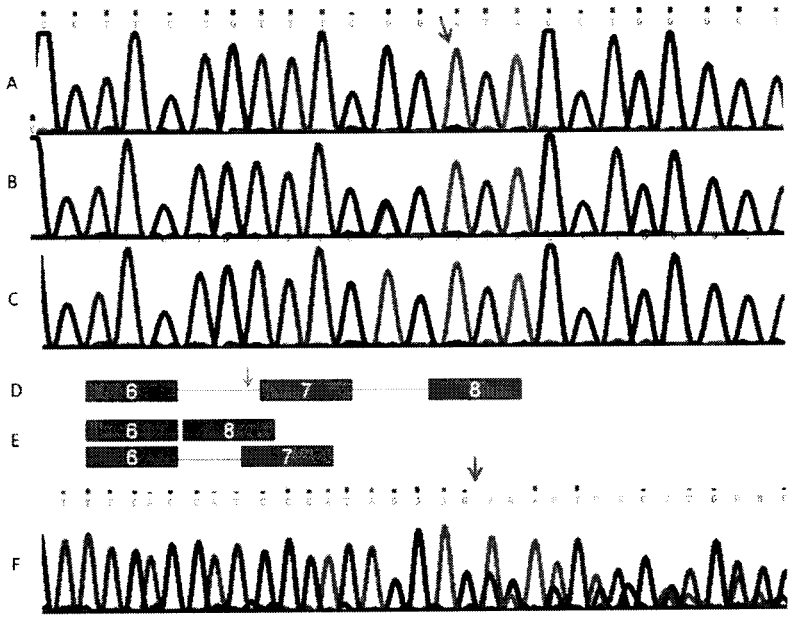

Figure 1. The c.801 −2 A−>G mutation in the DNAJC6 gene. The green arrow points at the first nucleotide of exon 7 and the mutation affects the preceding AG splice acceptor site of intron 6 which is changed to GG in the patient (A). The sequence of an obligate heterozygote is shown in (B) and that of a control in (C). Schematic representation of the mutation site at the genomic level (D) and its impact on the cDNA (E). Chromatogram of cDNA from a patient encompassing the 3′ junction of exon 6 (F) and demonstrating a transcript lacking exon 7 and another transcript where nNext to the last base of exon 6 (blue arrow) overlapping exon 8 sequence is the intronic sequences from intron 6 (c.801 −91). The normal exon 6/exon 7 spliced form is undetectable.
doi:10.1371/journal.pone.0036458.g001

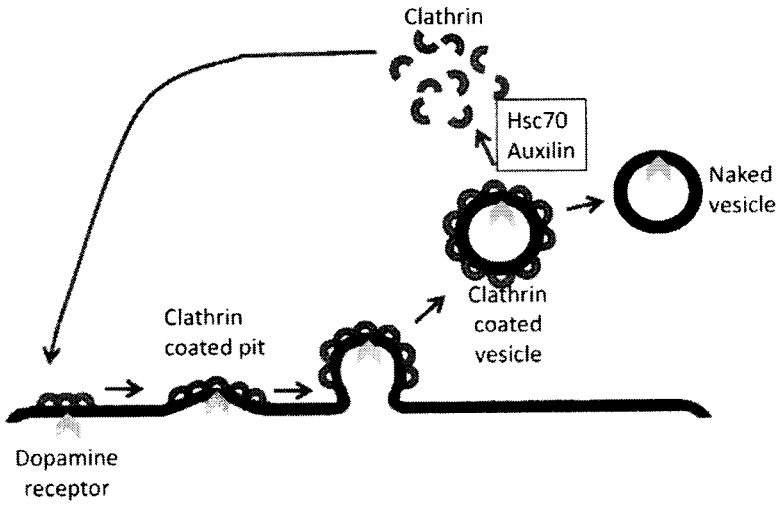

Figure 2. Schematic representation of clathrin-mediated endocytosis. Plasma membrane molecules (in this case the dopamine receptor) associate with nascent clathrin-coated pits which then mature invaginate and finally pinch off to form clathrin-coated vesicles. The shedding of the coat takes place after the vesicle buds from the plasma membrane. This process is driven by Hsc70 ATP hydrolysis activity which is recruited to clathrin coats by Auxilin. The uncoated vesicle fuses with the membrane of a target compartment and delivers its cargo. Clathrin molecules are directed to the plasma membrane for re-use.
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carboxyl-terminal J-domain (residues 886–931) which binds Hsc70 [10,11]. The low mRNA level in our patients and the production of two abnormal transcripts lacking either a significant part of the PTEN-like domain or the carboxyl-terminal J-domain, suggest that cells homozygous for the c.801-2 A−>G mutation lack the Auxilin protein.

Auxilin is selectively expressed in neurons and is enriched in nerve terminals [12]. In non-neuronal cells, another J-domain containing partner, the ubiquitously expressed cyclin-G-dependent kinase (GAK), co-chaperones Hsc70-mediated uncoating activity on CCV [13]. Reduction of Auxilin and its homologue GAK was shown to result in the impairment of CME and of the clathrin-dependent traffic of cargo from the Golgi to the lysosome [14]. Nonetheless, Auxilin is not redundant in mice and Auxilin null mice exhibit an abnormally increased retention of assembled clathrin on vesicles and in empty cages despite upregulation of GAK. This defect, in turn, leads to impaired synaptic vesicle recycling and perturbed CME, probably because of sequestration of clathrin coat components and their accessory factors with failure to form new clathrin-coated pits [15]. Mice lacking Auxilin have a high rate of unexplained early postnatal mortality, and surviving pups fail to thrive though they do have normal life span. We examined brain tissue of these Auxilin null mice but could not detect any alteration in substantia nigra morphology, dopamine transporter abundance or distribution (data not shown), in agreement with the lack of any gait or movement abnormalities in the mutant mice. Lack of neurodegeneration in transgenic mice that express mutated versions of PD genes is a recognized phenomenon in PD research [16].

The identification of the molecular basis of familial PD and juvenile variants is still in progress [17]. Loss of-function mutations in *PRKN*, *PINK1*, and *DJ-1*, all implicated in mitochondrial repair/elimination machinery, give rise to a pure, early onset PD. Another group of PD-causing genes participate in the endosomal/lysosomal pathway. α-synuclein, encoded by *SNCA*, was shown to participate in synaptic vesicle formation and recycling and to facilitate CME [18]. The *C. elegance* orthologue of LRRK2, another central player in PD pathogenesis, regulates the proper localization of synaptic vesicles in neurons [19]. Dominant mutations in PD patients were recently identified in *VPS35*, a component of the retromer complex which mediates retrograde transport between endosomes and the trans-Golgi network [20,21]. Another player in the endosomal/lysosomal compartment, ATP13A2, is also associated with PD [22]. Finally, mutations in the gene encoding the lysosomal enzyme glucocerebrosidase, which interacts with α-synuclein [23], have also been identified as PD susceptibility alleles [24]. The involvement of these five genes underscores the role of the endosomal/lysosomal pathway in PD pathogenesis. We propose that Auxilin is a new endosomal/lysosomal Parkinsonism-related gene. We refrain from the term "Parkinson Disease" since the patients did not respond to L-Dopa. Since dopamine receptors undergo CME [25] followed by endosomal sorting to recycling or degradation [26], it is conceivable that a homozygous deleterious mutation in the Auxilin encoding gene, *DNAJC6*, would give rise to abnormal dopamine receptor metabolism with the resultant parkinsonism. We studied the endosomal system in fibroblasts of patient II-2 by measuring transferrin uptake but observed no differences in either the levels or the pattern of transferrin uptake between control and mutant cells suggesting that in human fibroblasts Auxilin is redundant and its activity overlaps that of GAK.

DNAJC6 does not fall within any of the peaks discovered by recent PD genome-wide association studies. Focused studies on common variation in this gene are perhaps now warranted [27]. We determined the sequence of the coding exons and splice sites of *DNAJC6* in 15 patients with PD onset before 40 years of age but could not find any pathogenic mutation.

In summary, using homozygosity mapping in a consanguineous small family, followed by whole exome sequencing of a single patient who suffered from juvenile Parkinsonism, we were able to identify a new Parkinsonism-related gene. It is likely that *DNAJC6* is indispensible in the human substatia nigra but is redundant in peripheral tissues. Nevertheless, the findings presented above are

important for the genetic counseling of the patient's extended family, and emphasize the role of CME in the pathogenesis of PD.

Materials and Methods

Clinical description

The proband, patient II-4, presented at 13 years of age along with his 18 year old brother, patient II-2.The two brothers were the sons of first cousin Arab-Moslem parents of Palestinian origin (figure 3). The parents and three siblings were healthy. Pregnancy, delivery and early psychomotor development of the two patients was normal. On physical examination at 11 years, patient II-4 was first noted to have debilitating bradykinesia, rigidity, postural instability, hypomimia, and asymmetric tremor at rest. Therapeutic trials with Amantadine, Pramipexole, and L-Dopa did not provide any relief and the patient became wheel-chair bound at age 13. Despite being cognitively normal he could not attend school due to his physical impairment. Patient II-2 was reported to suffer from identical symptoms during childhood and had deteriorated to a dependent state by age 18. The course of his disease was more insidious with bradykinesia noted at 7 years and later appearance of rigidity, tremor and postural instability. The physical examination of both patients revealed hypomimia, slow and dysarthric speech, bradykinesis, pill-rolling tremor at rest, and postural instability with inability to walk. Glabellar tap was unextinguishable and no gaze paresis was elicited. In patient II-2 hypometric saccades were noted. Tone was increased in limbs with no spasticity, pyramidal signs or dystonia. Deep tendon reflexes were symmetric and normal. Plantar reflexes were downgoing. Fine alternating movements, finger and foot tapping were slow and reduced in amplitude. No cerebellar or sensory deficits were found. Brain Magnetic Resonance Imaging (MRI) was unremarkable in both patients. The parents, another brother and two sisters were healthy at ages 11 to 21 and had normal neurologic examinations.

The study was approved by the Hadassah Institutional Review Board and the Ministry of Health. The parents consented to participate.

Genetic mapping

Single nucleotide polymorphism (SNP) genotyping was performed in the DNA samples of the two patients and an unaffected brother, with the Affymetrix GeneChip Human Mapping 250 K Nsp Array as previously described [28]. Homozygous regions larger than 2 Mb were manually searched. Selected SNP markers were used for genotyping the remaining family members. The carrier rate of the pathogenic mutation was determined by Sanger sequencing of the relevant exon in DNA samples of 133 anonymous ethnic matched controls.

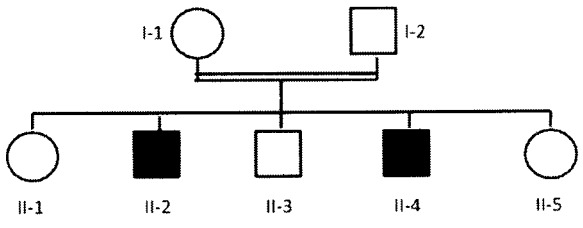

Figure 3. Family pedigree. The patients are represented by filled symbols.
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Exome sequencing

The DNA sample of patient II-2 was enriched for exonic sequences with the SureSelect Human All Exon v.2 Kit, which targets 44 Mb (Agilent, Santa Clara, CA, USA). Sequencing was carried out on a GAIIx (Illumina, San Diego, CA, USA) with 100-bp paired-end runs. Image analysis and base calling were performed with the Genome Analyzer Pipeline version 1.5 using default parameters. The sequence reads were aligned to human genome assembly hg18 (GRCh36) with DNAnexus software (Palo Alto, CA) using default parameters.

mRNA quantification

Total RNA was isolated from fibroblasts of patient II-4 and from five normal unrelated adult controls, using Tri Reagent (Sigma). DNA traces were removed by treatment with TURBO DNAse kit (Ambion). RNA was reversed transcribed using Improm II kit (Ambion) and random hexamers primers. DNA concentration of two plasmids, one with an ACTB insert and the other with a cDNA insert encompassing exon 4 and 5 of DNAJC6, was determined by spectrophotometer. Serial dilution of the plasmids' DNA was performed in order to create a calibration curve on real time PCR instrument (ABI 7900). These calibration curves were used to determine the copy number of the respective transcripts in RNA samples from the patient and the controls. We used the concentration of the ACTB cDNA to normalize the concentration of the DNAJC6 cDNA and each sample was PCR four times.

Transferrin uptake in fibroblasts

In order to study the endosomal system, transferrin uptake was measured in fibroblasts of patient II-2 as previously described [14] with some modifications. Cells were incubated for 1 h at 37°C in labeling medium (F12 containing 10 mM HEPES, pH 7.3 and 0.2% w/v BSA) to remove unlabeled transferrin. They were then labeled for 1 h on ice with 50 µg/ml Alexa fluor 488-transferrin (Invitrogen) in labeling medium. After rinsing twice with warm labeling medium, they were incubated at 37°C for either 20 or 40 min to allow transferrin uptake. The cells were then fixed with 2% formaldehyde in PBS (RT, 30 min) and examined with a Zeiss Axiovert 200 microscope equipped with a 100× oil immersion objective.

Web resources

Online Mendelian Inheritance in Man (OMIM) http://www.omim.org/ SeattleSeq Annotation website - http://snp.gs.washington.edu/SeattleSeqAnnotation/ PolyPhen-2 prediction of functional effects of human nsSNPs http://coot.embl.de/PolyPhen/ SIFT - Sorting Tolerant From Intolerant http://sift.jcvi.org/ Mutation taster - http://www.mutationtaster.org/ NHLBI Exome Sequencing Project Exome Variant Server - http://evs.gs.washington.edu/EVS/.

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Author Contributions

Conceived and designed the experiments: SE YC AS SL AB AT KHK LEG OE. Performed the experiments: YC ATS AS YTY SZ AT. Analyzed the data: SE YC ATS AS YTY SZ CJ AB AT KHK LEG OE. Contributed reagents/materials/analysis tools: CJ SL KHK LEG. Wrote the paper: SE YC AT KHK LEG OE. Managed patients, collected samples and delineated the phenotype: SE.

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DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability

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ABSTRACT

Familial parkinson's disease is both clinically and genetically heterogeneous. By mapping the disease locus with a lod score of 5.13 to $a < 3.5$ Mbp region at 1p31.3 in a consanguineous family and subsequent exome sequencing analysis, we identified homozygous truncating mutation p.Q734X in *DNAJC6*. Four members of the family were afflicted with juvenile parkinsonism that presented with mental retardation, pyramidal signs and epilepsy, as well as varying degrees of a progressive neurological disease. Recently a splicing mutation in the same gene was reported in two brothers with juvenile parkinsonism that was not L-Dopa responsive and not accompanied by pyramidal signs or mental retardation. Also, an 80-kb deletion that included *DNAJC6* sequences was identified in a boy reported as having obesity, epilepsy and mental retardation but not any signs of parkinsonism. The phenotype of our study family resembles both of those families, which among themselves do not share any clinical features. Our findings further establish *DNAJC6* as a juvenile parkinsonism gene, and expand the spectrums of the parkinsonism phenotype and *DNAJC6* mutation.

DNAJC6 encodes the neuronal co-chaperone auxilin. We found that its transcript is highly significantly more abundant in brain as compared to the non-neural tissues assayed.

1. Introduction

Parkinson's disease (PD) is characterized by resting tremor, muscular rigidity, bradykinesia, postural instability, and response to L-Dopa. Juvenile parkinsonism is described as parkinsonism with an age of onset <21 years. It is a rare and heterogeneous syndrome that can present with a clinical picture similar to idiopathic PD or manifest with additional signs, such as dystonia, cognitive impairment, neurobehavioral abnormalities, pyramidal disturbances, ophthalmoparesis, and autonomic dysfunction [1]. Recessive mutations have been identified in several genes associated with juvenile or young onset parkinsonism [2–7], three of which are responsible for clinical phenotypes that are almost indistinguishable from sporadic PD: *PARK2* (MIM 602544), which encodes parkin; *PINK1* (MIM 608309), which is responsible for PARK6; *DJ1* (MIM 602533), which is responsible for PARK7. Mutations in *PARK2* have been implicated in about half of patients with autosomal recessive early onset parkinsonism [2]. Mutations in *ATP13A2* (MIM 610513), responsible for PARK9 or Kufor-Rakeb syndrome, have been identified in patients with early onset parkinsonism, ophthalmoparesis, myoclonic jerks, pyramidal tract signs, and dementia [6,7]. PARK15 is a parkinsonian-pyramidal syndrome caused by *FBXO7* mutation (MIM 605648) and characterized by L-Dopa responsiveness, hyperreflexia, spasticity, dystonic posture, and normal mental status [5]. Other genes associated with similar clinical phenotypes include *PLA2G6* (MIM 603604) and *SPATACSIN* (*SPG11*; MIM 610844) [7].

Recently a homozygous splicing *DNAJC6* mutation was reported in two brothers with juvenile parkinsonism, via autozygosity analysis and exome sequencing [8]. Also, a homozygous 80-kb deletion that encompassed part of *DNAJC6* and part of *LEPR (leptin receptor)* was identified by oligonucleotide array-CGH in a boy having obesity, epilepsy and mental retardation but not any signs of parkinsonism, and the phenotype of the *DNAJC6* defect was suggested as mental retardation and/or epilepsy [9].

DNAJC6 (DNAJ/HSP40 homolog, subfamily C member 6; MIM 608375) encodes auxilin, which was originally identified as a clathrin-associated protein that was neuron-specific and enriched in nerve terminals, suggesting that it may play a role in synaptic vesicle recycling [10]. Auxilin is the co-chaperone that recruits HSC70 to the clathrin coated vesicles (CCVs) for disassembly [11–17]. Defective auxilin causing parkinsonism is in line with parkinsonism often being an endosomal disorder commonly related to CCVs and synaptic vesicle recycling. An association between SNP

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The Sac1 domain of *SYNJ1* identified mutated in a family with early-onset progressive parkinsonism with generalized seizures

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Abstract

This study aimed to elucidate the genetic causes underlying early-onset parkinsonism (EOP) in a consanguineous Iranian family. To attain this, homozygosity mapping and whole-exome sequencing were performed. As a result, a homozygous mutation (c.773G>A; p.Arg258Gln) lying within the NH_2-terminal Sac1-like inositol phosphatase domain of polyphosphoinositide phosphatase synaptojanin 1 (SYNJ1), which has been implicated in the regulation of endocytic traffic at synapses, was identified as the disease-segregating mutation. This mutation impaired the phosphatase activity SYNJ1 against its Sac1 domain substrates *in vitro*. We concluded that the *SYNJ1* mutation identified here is responsible for the EOP phenotype seen in our patients probably due to deficiencies in its phosphatase activity and consequent impairment of its synaptic functions. Our finding not only opens new avenues of investigation in the synaptic dysfunction mechanisms associated with parkinsonism, but also suggests phosphoinositide metabolism as a novel therapeutic target for parkinsonism.

Keywords

Homozygosity mapping; whole exome sequencing; SYNJ1; autosomal recessive parkinsonism

Introduction

Early-onset parkinsonism (EOP) is characterized by the presentation at a young age of tremor, hypokinesia, muscular rigidity, and postural instability (Jankovic, 2008). Parkinsonism may have many etiologies, including metabolic or infectious diseases, pharmacotherapy, or genetic causes. During the past years a number of inherited forms, which are usually characterized by juvenile onset, L-dopa responsiveness, and recessive pattern of inheritance, have been described (Martin, et al., 2011; Paisan-Ruiz, et al., 2010). To date, there are at least six different genes associated with autosomal recessive early-onset parkinsonism (AR-EOP): PARK2 (*PRKN*; MIM# 600116), PARK6 (*PINK1*; MIM# 605909), PARK7 (*DJ-1*; MIM# 602533), PARK9 (*ATP13A2*; MIM# 606693), PARK14 (*PLA2G6*; MIM# 612953), and PARK15 (*FBXO7;* MIM# 260300) (Hardy, et al., 2009). Mutations in *PRKN*, *PINK1*, and *DJ-1* cause more typical parkinsonism while *ATP13A2*, *PLA2G6*, and *FBXO7* mutations are responsible for complex syndromes, with pyramidal signs, cognitive decline, as well as other neurological and psychiatric symptoms, and may become non-responsive to levodopa treatment with the disease progression (Paisan-Ruiz, et al., 2010). Additionally, *PRKRA* (DYT16; MIM# 612067) and Spactasin (SPG11; MIM# 610844) mutations have also been reported in juvenile atypical parkinsonism forms and *SLC6A3* mutations are known to cause infantile dystonia-parkinsonism syndrome (PKDYS; MIM# 613135) (Camargos, et al., 2008; Kurian, et al., 2009; Paisan-Ruiz, et al., 2010). More recently, mutations within *DNAJC6* (MIM# 608375) encoding auxilin, a protein implicated in clathrin-mediated endocytosis, have been identified in families featuring autosomal recessive juvenile parkinsonism (Edvardson, et al., 2012; Koroglu, et al., 2013).

In the current study we aimed to identify the genetic causes underlying disease in a consanguineous family of Iranian ancestry featuring early-onset parkinsonism with generalized seizures. Whole exome sequencing (WES), particularly in conjunction with other methods like SNPs-based arrays, has become a fruitful strategy for gene identification in both complex and Mendelian traits and as such is an ideal approach to identify disease causal alleles (Krebs and Paisan-Ruiz, 2012; Singleton, 2011). Therefore, HM through high-throughput SNP genotyping followed by WES and disease-segregation analyses was performed for disease-locus and gene identification. As a result, we identified a disease-segregating mutation, c.773G>A causing p.Arg258Gln, within the NH_2-terminal SAC1-like inositol phosphatase (Sac1) domain of the polyphosphoinositide phoshatase SYNJ1 (MIM#

604297), which also contains an inositol 5-phosphatase domain and a COOH-terminal proline-rich region that interacts with a variety of SH3 domain-containing endocytic factors, such as endophilin and amphiphysin (Dittman and Ryan, 2009). We later evaluated the effects of the p.Arg258Gln mutation on the enzymatic properties of SYNJ1 and concluded that the *SYNJ1* p.Arg258Gln mutation impairs the Sac1-like phosphatase activity. Our current finding not only opens a new avenue of investigation in the field of Parkinson research, but also adds new evidence that defects in membrane traffic at the synapse may be key factors in the development of both Parkinson disease and parkinsonism.

Methods

Subjects

A consanguineous family featuring early-onset parkinsonism was clinically examined. The family consisted of healthy parents, who were first-degree relatives, two affected and three unaffected siblings (Figure 1). The local ethics committee at Tehran University of Medical Sciences approved this study and informed consent was obtained from all participants. DNA samples from all members were isolated from whole blood using standard procedures.

Genetic variants identified within disease-associated loci (Tables 1 and 2) were additionally tested by direct Sanger sequencing in 96 DNA samples belonging to controls individuals of Iranian ancestry from the same geographical region of our family and 92 DNA samples of Caucasian neurologically normal individuals available to purchase at Coriell Cell Repository (NDPT093; http://www.coriell.org/).

20 DNA samples of affected individuals with early-onset parkinsonism were also tested for the entire coding region of *SYNJ1*. Although seizures were not present in any of these patients, all presented with complex early-onset parkinsonism, including parkinsonism, spasticity, and dystonia as common phenotypic features.

Genome-wide SNP Genotyping and Homozygosity Mapping

SNP genotyping was performed in all available family members (n=7) using the HumanOmniExpress beadchips and HiScanSQ system (Illumina Inc., San Diego, CA, USA). Genotyping quality assessments were undertaken according to the appropriate options within the Genome Studio program (GS; Illumina). PLINK input reports were generated within the GS and uploaded to PLINK v1.07 program (Purcell, et al., 2007). Homozygous segments were identified using the ROH tool (Runs of homozygosity) within PLINK, where a minimum physical size threshold of 1 Mb and at least 100 homozygous adjacent markers in length, including no more than two SNPs with missing genotypes and only one possible heterozygous genotype, were used as inclusion criteria. Subsequently, overlapping and potentially matching segments were also identified in PLINK using an allelic matching of 0.99 as threshold. Homozygous segments were also visualized using the Illumina genome viewer (IGV) within the GS program.

Whole Exome Sequencing

Whole exome sequencing was performed in both affected siblings. The SureSelect Human All exon 50Mb exon-capture kit was used for library enrichment (Agilent Technologies Inc., Santa Clara, CA, USA). The captured exome libraries were then sequenced on a HiSeq2000 according to the manufacturer's instructions for paired-end 100-bp reads (Illumina Inc. San Diego, CA, USA) and on a single flow cell lane. After sequencing, data were put through a computational pipeline for WES data processing and analysis following the general workflow adopted by the 1000 genomes project (DePristo, et al., 2011). First, the alignment of raw sequence reads to the human reference genome sequence (NCBI GRCh37) was

performed using a fast lightweight Burrows-Wheeler Alignment Tool (BWA) (Li and Durbin, 2009). The Genome Analysis Toolkit (GATK v1.5-16-g58245bf) was then used for base-quality recalibration and local realignment to minimize base calling error and mapping error, respectively. Lastly, the GATK Unified Genotyper tool was employed to call single-nucleotide substitutions (SNP/SNV) and short insertions/deletions (INDEL). Only passing variants were included in the final variant set. Calls were filtered based on the mapping quality (q30 or higher) and depth of coverage (d10 or higher). Resulting calls were annotated with AnnTools, an exhaustive genome annotation toolkit (Makarov, et al., 2011).

Filtering of Common Genetic Variation

Any potential mutation observed as common variation (frequency > 5%) in the dbSNP137 or 1000 Genomes Project Phase 1 was removed for further analyses. Genetic variants mapping to intra-genic, intronic, and non-coding exonic regions, with the exception of those variants mapping close to splice sites, were also removed since they are unlikely to be causative. It is worth noting that in the 1,000 Genome project five major populations, including West African, European, American, and East and South Asian, are targeted. Then, a second filter, which consisted of removing common variation (frequency > 5%) present in other public databases, such as the Exome Variant Server of the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) (Exome Variant Server, 2012), exomes generated in house from unaffected members of families with various phenotypes (n=40), and exome data generated in 500 Caucasian control samples that were obtained as VCF files from a large collection of unaffected Caucasian individuals that have been sequenced as part of the ARRA Autism Sequencing Collaboration (AASC) (MH089025, Mark Daly, communicating PI, Joseph D Buxbaum, Bernie Devlin, Richard Gibbs, Gerard Schellenberg, James Sutcliffe, collaborating PI's), was applied to all novel coding genetic mutations found to be present in both siblings. Only variants that we know for certain that were not pathogenic were included in this second filter. This filter was done last because low frequency genetic alleles could not have been removed, since, for example, low frequency heterozygous alleles may cause a disease when present in the homozygous state.

Prediction of Mutation Pathogenicity

To assist in causative gene identification, the pathogenicity of each novel mutation identified present in both siblings and absent in large number of control individuals was predicted by two computational methods previously evaluated as most efficient (Thusberg, et al., 2011): MutPred (http://mutpred.mutdb.org/) and SNPs&GO (http://snps-and-go.biocomp.unibo.it/snps-and-go/). All sequence variant descriptions were also examined using the Mutalyzer program (http://www.LOVD.nl/mutalyzer/). The HomoloGene database from NCBI web site was also used to examine the conservation of both disease-segregating mutations in different species (http://www.ncbi.nlm.nih.gov/homologene). Clustalw2 was also used to align Synaptojanin 1 with other human proteins containing Sac1 domains (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Gene Screening Analyses

Genomic primers for PCR amplifications were designed using a primer design public website (http://ihg.gsf.de/ihg/ExonPrimer.html). Primers were used to amplify exon 7 of *MCM4*, exon 3 of *SPATA31A1* (previous symbol, *FAM75A1*), and all coding exons of *SYNJ1* (primer sequences available upon request). PCR amplifications were performed as previously described (Karkheiran, et al., 2012). All purified PCR products were then sequenced in both forward and reverse directions with Applied Biosystems BigDye terminator v3.1 sequencing chemistry as per the manufacturer's instructions. The resulting sequencing reactions were resolved on an ABI3130 genetic analyzer (Applied Biosytems,

Foster city, CA, USA) and analyzed using Sequencer 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Mutagenesis and Protein Expression and Purification

Direct mutagenesis was done using the QuikChange Lightning Site-Directed Mutagenesis kit (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA), both pcDNA3-FLAG-human-Synaptojanin 1-145 and pEGFPC1-FLAG-human-Synaptojanin 1-145 (Addgene: http://www.addgene.org/22291/ and http://www.addgene.org/22293/

and dysarthria. He was treated with trihexyphenidyl and l-dopa/carbidopa with improvement in both tremor and bradykinesia but soon developed l-dopa-induced dyskinesias at very low doses (50mg). Due to severity of dyskinesia l-dopa was stopped and he was treated with the dopamine agonist bromocriptine up to 15 mg/day with modest improvement of all parkinsonian symptoms and a reduction in dyskinesia. There was no sign of cognitive impairment. He continued taking phenobarbital for over 20 years with acceptable seizure control until three years ago; he now only has one to two seizures per year.

On physical examination at the age of 29, he had generalized bradykinesia with bilateral limb rigidity, right side predominant resting tremor, chin tremor, and inability to open his eyelids spontaneously. He had jerky saccades and saccadic pursuits. His speech was unintelligible due to severe hypophonia and his gait was shuffling with postural instability. Off medication Unified Parkinson Disease Rating Scale part III (UPDRS-III) score was 38 (maximum score = 56) with most scores related to bradykinesia and rigidity. Muscle strength, deep tendon reflexes, cerebellar, and sensory functions were all normal. Administration of 25mg/day of l-dopa completely alleviated the ALO and improved bradykinesia, rigidity, oculomotor abnormalities, and shuffling gait. One hour after medication administration, his gait was normal, his speech became more intelligible, but postural instability was unchanged. Wearing off started to appear 2 hours after drug administration (The UPDRS-III scores were 28 and 18 at 30 minutes and 1 hour respectively after taking levodopa, the latter being mostly due to rest tremor). With doses greater than 25 mg of levodopa, in addition to dyskinesias, the patient became euphoric and talkative. There was no evidence of hyposmia, urinary urgency, incontinence, erectile dysfunction, constipation, or orthostatic hypotension. During the "on" period, cognitive functioning was intact with moderate bradyphrenia. Brain MRI showed mild cortical atrophy and bilateral symmetric T2 hyperintensities in the white matter, mostly posteriorly (Figure 1a, b, c).

Patient II—This 39-year-old woman had a febrile convulsion in infancy followed by unprovoked generalized tonic-clonic seizures for which she was treated with phenobarbital. Developmental milestones were normal. In her early twenties she developed a right hand tremor, followed by bradykinesia, severe jaw tremor, and eyelid twitching. The disease progression was similar to that of her brother. She could walk unassisted until the age of 32 years, but subsequently required assistance. She had severe dyskinesia with small doses of levodopa (25 mg) and non-ergot dopamine agonists, and only tolerated anticholinergics and bromocriptine, which gave her good tremor control and moderate improvement in bradykinesia. Cognitive function was grossly normal. Brain MRI performed at 37 years old showed a large foramen magnum meningioma (Figure 1d, e, f). After surgery to remove this tumor, the patient lost her ability to walk and became bedbound. During her last visit, at the age of 39, she was anarthric and in fixed "quadriplegia in flexion" posture, but she correctly responded to "open and close your eyes" and "make a sound" commands. Mass lesions compressing the brainstem may cause parkinsonism; however, the *SYNJ1* mutation, shared with her brother, and not this lesion, are likely to be responsible for our patient's clinical syndrome. The absence of localizing corticospinal signs and the lack of surrounding edema seen on MRI indicate that the tumor grew extremely slowly, displacing the brainstem without causing significant neuronal dysfunction. The patient's seizures could not be attributed to this tumor. Although not illustrated here, other views of the brain MRI clearly show that there was no evidence of obstruction of cerebrospinal fluid flow through the aqueduct and the lateral ventricles were of normal size. In addition, the features of parkinsonism seen here are not observed in ventricular obstruction.

Molecular Analyses

All available family members (n=7) were subject to genome-wide SNP genotyped analyses and HM, which revealed three potential disease-associated loci. These loci, only shared by the two affected siblings, were located at chromosomes 3, 8, and 21 (Table 1). No shared region of homozygosity or copy number variation (CNV) was identified within the known PD loci. The WES performed in the two affected siblings captured 95.18% and 95.88% of the target exome at 20-fold coverage or higher for patients I and II, respectively. This lead us to the high-quality identification of 61,286 SNVs and 9,836 indels for Patient I and 67,337 SNVs and 13,061 indels for Patient II. All known PD genes were well covered by our WES approaches and excluded. After an adequate filtering (see methods), 832 non-synonymous, 11 nonsense, and 459 synonymous genetic variants were identified for Patient I, while 982 non-synonymous, 10 nonsense, and 463 synonymous genetic variants were identified for Patient II. None of the nonsense genetic variants were present in both patients and only 223 non-synonymous genetic variants (187 heterozygous and 36 homozygous) were common to both affected siblings. Further investigation of the 223 novel genetic variants in the dbSNP137, in other public databases, and exomes generated in house (see Methods) left us with only three novel genetic variants present in both siblings (Table 2). Two of these, c.706A>G (p.Thr236Ala) within minichromosome maintenance complex component 4 (*MCM4*; 8q11.2) and c.773G>A (p.Arg258Gln) within synaptojanin 1 (*SYNJ1*; 21q22.2), were located in genomic regions previously found associated with disease through homozygosity mapping (Tables 1 & 2). The genotyping area harboring the third variant (*SPATA31A1* locus) was additionally inspected, but only a small homozygous area of 91kb also shared by unaffected individual was identified. As expected, subsequent sequencing of this variant in all family members revealed that it does not segregate with disease. Both variants located within the disease-associated loci were subsequently validated through Sanger sequencing and examined in the remaining family members: both mutations were present in the homozygous state in both affected siblings while both parents where heterozygous mutation carriers and unaffected members were either homozygous carriers for the wild-type allele or heterozygous mutation carriers, clearly consistent with a disease-segregation status. Further testing of both mutations in 376 control chromosomes (184 Caucasians and 192 Iranians) failed to identify any additional mutation carrier. However it is very unlikely that the phenotype observed in our patients is due to the p.Thr236Ala mutation in *MCM4*, which is essential for the initiation of eukaryotic genome replication, since it was predicted to be non-causative and *MCM4* mutations have recently been associated with clinical features, such as autosomal recessive adrenal insufficiency, short stature, natural killer cell deficiency, and familial glucocorticoid deficiency (Casey, et al., 2013; Hughes, et al., 2012; Yamaguchi, et al., 2013), not present in our patients (Table 2). Moreover, none of the patients already described with pathogenic *MCM4* mutations showed signs of parkinsonism; and although none of the *MCM4* mutations identified, including the one described here, lie within a functional domain, they all seem to be located throughout the protein, ruling out the possibility that *MCM4*-associated phenotypes are determined by the localization of the mutation within the protein. By contrast, the p.Arg258Gln mutation in *SYNJ1*, a highly brain enriched polyphosphoinositide phoshatase involved in synaptic vesicle recycling (Cremona, et al., 1999; McPherson, et al., 1996), was predicted to be pathogenic. This amino-acid change results from a G>A mutation at position 258 for transcripts 1 (NM_003895.3) and 2 (NM_203446.2) and 219 for transcripts 3 (NM_001160302.1) and 4 (NM_001160306.1). *SYNJ1* comprises two inositol phosphatase domains arranged in tandem – an N-terminal Sac1 inositol phosphatase domain and a central inositol 5-phosphatase domain – followed by a COOH-terminal proline-rich region that interacts with a variety of protein factors implicated in signaling, endocytosis and actin nucleation (McPherson, et al., 1996; Slepnev and De Camilli, 2000). The Sac 1 domain dephosphorylates PI3P, PI4P, and PI(3,5)P_2 (Guo, et al., 1999), while the preferred

substrates of the 5-phosphatase domain are $PI(4,5)P_2$ and $PI(3,4,5)P_3$ (Cremona, et al., 1999). The p.R219/258 amino-acid is localized in the Sac1 domain and is highly conserved among 18 different orthologs and in the SYNJ2 protein (NM_003889), which has a similar domain structure but more widespread tissue distribution than SYNJ1(Nemoto, et al., 1997) (Figure 1); it is also conserved among three other human proteins containing Sac1 domains, including SACM1L (SAC1, NM_014016.3), INPP5F (SAC2, NM_014937), and FIG4 (SAC3, NM_014845) proteins (data not shown). Taking into account all public SNPs databases, exomes generated in house, and DNA control samples testing by direct Sanger sequencing, p.Arg258Gln was absent in over 10,000 control chromosomes, likely supporting its pathogenicity.

Although 20 DNA samples of affected individuals with complex early-onset parkinsonism were additionally tested for the entire coding region of *SYNJ1*, we failed to identify any other mutation carrier.

We thus tested whether the p.Arg258Gln (p.Arg219Gln) mutation impairs the phosphoinositide phosphatase activity of the Sac1 domain. The c.1149T>A/c.1264T>A (p.Cys383Ser/p.Cys422Ser) mutation in the Sac1 domain of human SYNJ1 domain was used as a control, based on previous work showing that p.Cys383Ser impairs the Sac1-like phosphatase activity towards PI3P and PI4P (Mani, et al., 2007). This analysis showed that while p.Cys383Ser (p.Cys422Ser) and p.Arg219Gln (p.Arg258Gln) mutations did not affect in a significant way the phosphatase activity against $PI(4,5)P_2$, which is known to be accounted by the 5-phosphatase domain of SYNJ1, they drastically inhibited the phosphatase activity against PI3P and PI4P (Figure 2c), as expected for mutations that impair the Sac1-like phosphatase activity of this enzyme (Guo, et al., 1999).

Discussion

We report on a consanguineous family with autosomal recessive early-onset parkinsonism with generalized seizures. Two siblings developed seizures in early childhood, followed by resting hand tremor and bradykinesia in their early 20's, and progressing to chin tremor and apraxia of eyelid opening. HM, WES, and gene screening analyses led us to the identification of three disease-associated loci and two disease-segregating genetic variants (Tables 1 and 2). Both disease-segregating variants, p.Thr236Ala and p.Arg258Gln, were absent in controls, including some of Iranian ancestry. Although the Iranian control population tested here was not large, given the high rate of consanguinity extant in the Iranian population (Akrami and Osati, 2007), the prevalence of a rare private allele in this population is much higher than the generally expected (Bittles and Black, 2010). Moreover, our follow up investigations in the most likely disease-causing mutation and the only one predicted to be pathogenic, provided additional evidences to support the p.Arg258Cys mutation as the disease-causing mutation. Previous studies demonstrated that the synaptic function of SYNJ1 depends on its intact dual phosphatase activity (Mani, et al., 2007). Here we showed that the disease-linked mutation almost entirely eliminated both 3-phosphatase and 4-phosphatase activities (Figure 2c), which also suggests that the patients carrying this mutation may have increased levels of SYNJ1 substrates. The inhibition of the Sac-1 activity is expected to impair synaptic vesicle endocytosis and reavailability in nerve terminals, as suggested by previous reports and studies done in mice (Cremona, et al., 1999; Mani, et al., 2007).

The identification of a *SYNJ1* mutation linked to parkisonism is interesting because this lipid metabolizing enzyme is a key regulator of phosphoinositide metabolism in the nervous system and has been shown to regulate key synaptic processes, such as the recycling of synaptic vesicles, the internalization of AMPA receptors, as well as actin dynamics (Frere,

et al., 2012; Gad, et al., 2000; Gong and De Camilli, 2008; Harris, et al., 2000; Verstreken, et al., 2003). Although *SYNJ1* mutations have not been previously associated with a human neurological disease, the overexpression of this protein in Down syndrome mouse models causes a deficiency of $PI(4,5)P_2$ as well as learning deficits (Voronov, et al., 2008), while Synj1 haploinsufficiency is protective against the synaptotoxic action of beta-amyloid *in vitro* and in mouse models of Alzheimer disease (Berman, et al., 2008; McIntire, et al., 2012). Additionally, *Synj1* knockout mice fail to thrive and rapidly develop severe neurological manifestations including severe weakness, ataxia, spontaneous epileptic seizures, and poor motor coordination (Cremona, et al., 1999); and loss of SYNJ function in *C. elegans*, zebrafish and *drosophila* results in severe nervous system defects mutants, such as abnormal balance, posture, and locomotion (Harris, et al., 2000; Minagawa, et al., 2001; Stefan, et al., 2002; Van Epps, et al., 2004; Verstreken, et al., 2003).

There is mounting evidence that synaptic vesicle trafficking pathways are implicated in neurodegeneration (Esposito, et al., 2012). Alpha-synuclein has been implicated in synaptic vesicle exocytosis and in synaptic vesicle recycling (Burre, et al., 2010; Nemani, et al., 2010). *LRRK2* mutations also cause a defect in synaptic vesicle endocytosis, which can be rescued by co-expression of Rab5b (Heo, et al., 2010). LRRK2 has also been identified as regulator of endophilin-A (Matta, et al., 2012), which is a major binding partner of SYNJ1 and interacts and participates with Parkin in the ubiquitination of proteins within synaptic endophilin-A complexes (Trempe, et al., 2009). And lastly, disruption of the SYNJ1/endophilin interaction by Cdk5, which phosphorylates parkin (Rubio de la Torre, et al., 2009; Yamamoto, et al., 2005) and SYNJ1 (Tan, et al., 2003), has been suggested to be important for synaptic vesicles recycling (Lee, et al., 2004).

These findings add further support to the evidence that many parkinsonism-associated proteins, including alpha-synuclein, parkin, dardarin (LRRK2), auxilin, and now also SYNJ1, act as important regulators of synaptic vesicle trafficking pathways at synapses. All together they foster the hypothesis that aberrant functions of proteins implicated in the SV recycling may be responsible for at least part of the neuronal cell loss seen in Parkinson disease and other neurodegenerative diseases.

In conclusion, our data support the disease-segregating p.Arg258Gln (p.Arg219Gln) mutation, which is absent in over 10,000 control chromosomes and impairs the Sac1-like domain activity, as the genetic cause responsible for autosomal recessive parkinsonism with generalized seizures in our patients. Although further studies are warranted to gain insights into the mechanisms by which dysfunction of phosphoinositide phosphatases, such as synaptojanin 1, results in neurological dysfunction and likely cell death, our current finding opens a new avenue of investigation in the field of Parkinson research, adds further evidence that defects in membrane trafficking at the synapse may be key factors in the development of both Parkinson disease and parkinsonism, and suggests phosphoinositide metabolism as a novel therapeutic target for parkinsonism.

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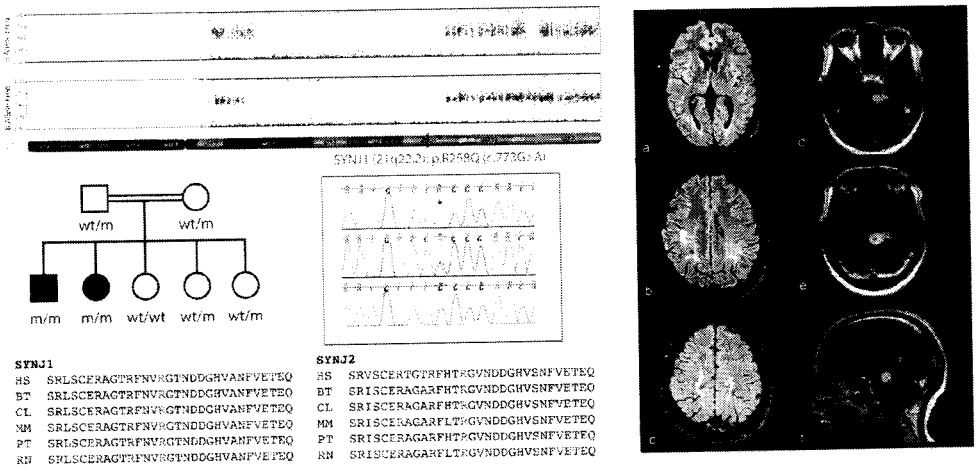

Figure 1.

Left side Upper panel: B allele frequency plots from Genome studio software (Illumina) showing the loss of heterozygosity area (LOH (blank areas); 21q21.1-22.2) shared by both affected siblings; the location of *SYNJ1* within the disease-associated LOH area is highlighted in red. Middle panel: The pedigree structure of the Iranian family with EOP is shown in the left side while the Sanger chromatograms of the human reference sequence (bottom) as well as both heterozygous (middle) and homozygous (top) mutant sequences are shown in the right side; a black arrow highlights the pathogenic mutation. The p.Arg258Gln mutation is represented as p.R258Q. Bottom panel shows the conservation of R258 amino-acid (highlighted in red) within different orthologs in both SYNJ1 and SYNJ2 proteins. HS: Homo sapiens; BT: Bos Taurus; CL: Canis lupus; MM: Mus musculus; PT: Pan troglodytes; RN: Rattus norvegicus. **Right side:** Brain FLAIR magnetic resonance images (MRIs) of patient 1 showing bilateral periventricular (a) and subcortical (b and c) white matter hyperintense signals predominantly in parietal-occipital regions. Axial (e & e) and sagittal (f) post-gadolinium brain MR images of patient 2 show an intensely enhancing meningioma with compressing the left pons and lower midbrain. There was no evidence of obstructive hydrocephalus.

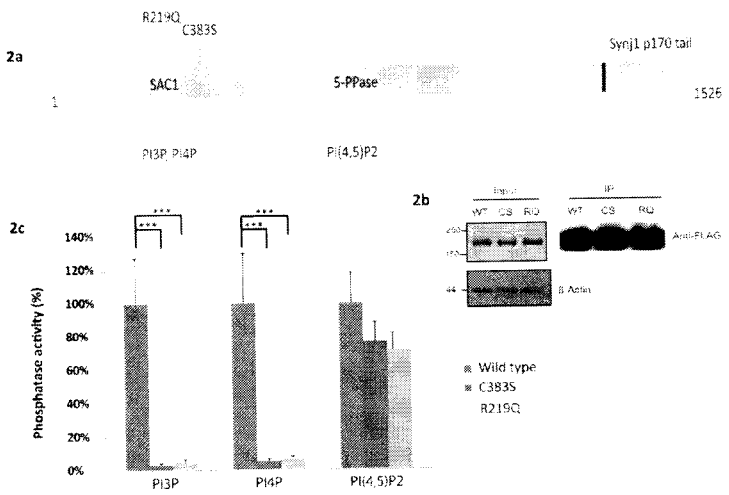

Figure 2.

2a: Domain organization of SYNJ1-145 (3) and SYNJ1-170 (4) isoforms (Haffner, et al., 1997; Perera, et al., 2006). SYNJ1-145 is highly expressed in the nervous system, specifically in mature brain and synapses, while the alternatively spliced isoform SYNJ1-170, which has a longer C-terminal, is ubiquitously expressed but at lower levels. The domain organization was drawn based on published reports and SMART predictions (http://smart.embl-heidelberg.de/) (Guo, et al., 1999; Jha, et al., 2004) and includes the two polyphosphoinositide phosphatase regions and the C-terminal region, which is responsible for protein-protein interactions. The extended C-terminal tail of SYNJ1-170 contains binding sites for proteins of the endocytic machinery. Both p.Arg219Gln (R219Q) and p.Cys383Ser (C383S) mutations lying in the Sac1-like region are shown. **2b:** Anti-Flag Western-Blot showing equal amounts of affinity purified Flag-tagged wild-type and mutant SYNJ1. **2c:** Wild-type and mutants SYNJ1 were expressed in HEK 293T cells and purified by immunoprecipitation. The enzymatic activity was measured by malachite-green based assays. Error bars are SEM. Graphics represent means and SEM of at least nine independent experiments. (***) indicates p values of $p \leq 0.001$.

Table 1

Homozygous segments found only present in both affected sibling by homozygosity mapping through genome-wide SNP genotyping

CHR	SNP1	SNP2	Position 1 (bp)	Position 2 (bp)	Size (Kb)	N° SNP
3	rs9871790	rs2046037	0	2,600,804	2600.8	1039
8	rs11993658	rs16938809	46,886,735	75,062,690	28176	6097
21	**rs171477**	**rs2834280**	**19,150,133**	**35,228,502**	**16078.4**	**4600**

Highlighted in bold is the genomic region turned out to be the disease-associated locus.